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Antimycobacterial activity of chemically defined natural substances from the Caribbean flora in Guadeloupe

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Abstract

Eight chemically defined, naturally occurring compounds were extracted from the tropical flora of the Caribbean island of Guadeloupe: pilocarpine, an alkaloid from *Pilocarpus racemosus*; heraclenol and isomeranzin, coumarins from *Triphasia trifolia*; lochnerin, an indole alkaloid from *Rauwolfia biauriculata*; ibogaine and voacangine, indole alkaloids from *Tabernaemontana citrifolia*; texalin, an oxazole from *Amyris elemifera*; and canellal, a sesquiterpene dialdehyde from *Canella winterana*. An essential oil fraction from *Canella winterana* was also tested. The antimycobacterial activity of these substances was tested against *Mycobacterium tuberculosis*, *M. avium* and *M. kansasii* using the Middlebrook 7H11 agar medium, the Bactec 460-TB radiometric methodology, and determination of bacterial viable counts. Three compounds, namely ibogaine, voacangine and texalin, showed antimycobacterial activity. Investigations on the structure-modification and structure-activity relationships of these compounds may help determine new targets for future drug development. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

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1. Introduction

The emergence of the AIDS pandemic has profoundly changed the picture of mycobacterial disease, as some 25–50% AIDS patients are infected with nontuberculous mycobacteria in the United States and Europe [1], whereas in other parts of the world such as sub-Saharan Africa and the Caribbean, *Mycobacterium tuberculosis* is an equally im-

portant opportunistic infection among AIDS patients [2]. The recent upsurge in the incidence of tuberculosis with significant emergence of multi-drug-resistant cases [3] has also focused attention on the priority of discovering effective new drugs.

Although herbal medicines such as Chaulmoogra oil and its purified esters from *Hydnocarpus* species have long been used for the treatment of leprosy [4], and a few reports have shown anti-*M. avium* activity of a crude extract of *Allium sativum* [5,6], the role of chemically defined substances from plants as antimycobacterial agents has not been established. In the

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present study, we investigated eight chemically defined natural substances from the tropical flora in the Caribbean island of Guadeloupe as well as an essential oil fraction, and report on their activity against *M. tuberculosis*, *M. avium* and *M. kansasii*, which have recently emerged as major opportunistic infections among AIDS patients [1,2].

2. Materials and methods

2.1. Plant material

The material was collected in Guadeloupe from plants shown in Table 1. Voucher specimens for Apocynaceae have been deposited at the herbarium of the National Museum of Natural History, Paris, which include *Rauwolfia biauiculata* J. Müll under the reference C. Sastre 2609, and *Tabernaemontana citrifolia* L. under the reference C. Sastre/J. Fournet 2652. Rutaceae specimens have been deposited at the Herbarium of the National Institute for Agricultural Research, Guadeloupe, and include *Triphasia trifolia* (F. Burm/P. Wilson) under the reference GuadFournet 1774, *Pilocarpus racemosus* var. *racemosa* under the reference GuadFournet 3376, and *Amyris elemifera* L. under the reference GuadFournet 4057. The Canellaceae *Canella winterana* L. Gaertn. was also deposited at the same institution under the reference GuadFournet 1867.

2.2. Natural compounds

Isomeranzin, heraclenol and canellal were obtained by chromatography of a chloroform extract of dried, powdered leaves as reported earlier [7]. Ibo-gaine, voacangine, lochnerin, pilocarpine and texalin were obtained as reported [8,9]. Briefly, the dried powdered material was moistened with 25% NH_4OH , and extracted by chloroform. The organic phase was treated with 2% HCl , the acid layer was washed with petroleum ether, basified with 25% NH_4OH , and extracted with chloroform. The organic phase was washed with water, and dried over Na_2SO_4 . The organic extracts were evaporated and subjected to chromatography on silica gel (reference 13895 and 5717 from Merck, Frankfurt, Germany). The essential oil fraction from *Canella winterana* was

obtained by the steam distillation of fresh leaves with a Clevenger type extractor. Detailed compound identification was performed using standard methodology, and included: ^{13}C and ^1H NMR spectra (measured on Bruker AM300 and AM400 from Bruker, Karlsruhe, Germany, respectively), UV and IR spectra (determined on Perkin Elmer apparatus from Perkin Elmer, Norwalk, CT, USA), and MS spectra (determined with a Kratos M550 from Kratos, Manchester, UK, run at 70 eV under a 8 kV tension). The chemical structures of various substances used in this investigation (except the essential oil of *Canella winterana*) are shown in Fig. 1. The main constituents of the essential oil from leaves of *Canella winterana* were noncyclic terpenoids: myrcene (68%), β -farnesene (8%), linalol (2%), and nerolidol (2%).

2.3. Organisms and growth

M. tuberculosis H37Rv, *M. avium* clinical isolate 733 from an AIDS patient, and *M. kansasii* clinical isolate 94-069 used in this investigation were from our own culture collection. The isolates were grown as fresh Löwenstein-Jensen (LJ) slants at 37°C.

2.4. Determination of minimum inhibitory concentrations (MICs)

MIC was defined as the lowest drug concentration that inhibited more than 99% of the bacterial population, and was determined using the 1% proportion method with Middlebrook 7H11 agar medium [10] or radiometrically using 7H12 broth at a pH of 6.8 ± 0.2 by the Bactec 460-TB methodology (Becton-Dickinson Diagnostics Instruments Systems, Sparks, MD, USA). For *M. tuberculosis* [11], 0.1 ml of a homogenized bacterial suspension adjusted to McFarland 1 with diluting fluid was injected into a Bactec 12B vial. This vial was used as a primary inoculum after the growth index (GI [10]) reached 500 as follows: 0.1 ml of bacterial suspension (about 10^4 CFU ml^{-1}) from the preculture vial was injected into drug-containing vials. The two controls included a first vial inoculated with the same number of organisms as the drug-containing vials, and a second control containing a 100-fold diluted initial bacterial inoculum (about 10^2 CFU ml^{-1}). Test and control vials were incubated at 37°C and the GI was re-

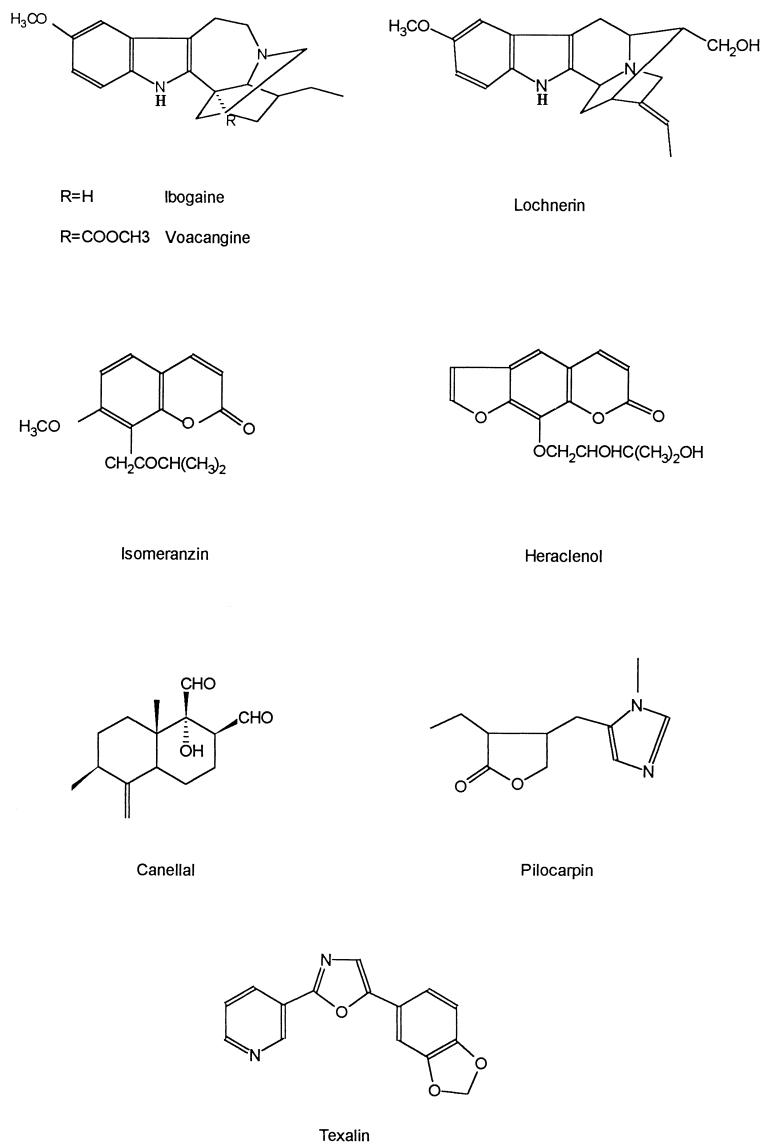


Fig. 1. Structure of various chemically defined compounds extracted from the tropical flora in Guadeloupe.

corded daily. When the GI of the 1:100 control vial reached 30, the test was read at least one additional day before it was terminated.

Due to more rapid growth of *M. avium* and *M. kansasii* in the Bactec system, the initial bacterial inoculum added in the Bactec vials depended on the species studied as described recently [12]. Briefly, after a preculture of a strain in an initial 12B Bactec vial to a GI of 500, the drug-containing vials were

inoculated with a 1:100 diluted preculture in case of *M. kansasii* and *M. avium* and compared to a control vial inoculated with 100 times less bacteria in the absence of the drug.

The results were interpreted as follows: if the difference in the GI values from the previous day (called Δ GI) for drug-containing vials was less than the Δ GI of the 1:100 control (and provided that the GI of the test vial did not exceed 50), then the drug

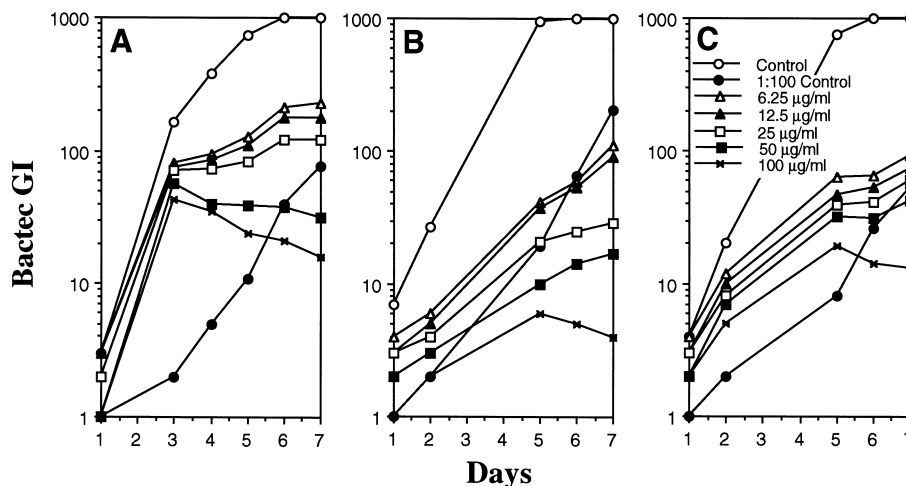


Fig. 2. Typical radiometric data illustrating the dose-dependent effect of voacangine (A), texalin (B), and ibogaine (C) against *M. tuberculosis*, *M. avium*, and *M. kansasii*, respectively.

concentration tested was considered to have inhibited more than 99% of the bacterial population and was designated MIC. Stock solutions of voacangine and texalin were prepared in a minimal volume of methanol while all other compounds were dissolved in a minimal volume of ethanol, followed by further dilutions directly in the 7H12 broth or the diluting fluid in both cases.

2.5. Determination of bacterial viability

Bacterial colony forming units (CFU) were deter-

mined by plating the bacterial suspensions from individual Bactec vials at the beginning and at the end of the experiments on 7H11 agar medium for viable count enumeration as reported previously [11,12]. Briefly, 0.1 ml of the culture from Bactec vials was taken and serially diluted 10-fold to provide successive dilutions ranging from 10^{-1} to 10^{-5} . Bactericidal activity was determined by plating 0.1 ml of aliquot from each of the 10-fold dilutions to 7H11 agar plates. CFU counts were assessed after 21 days of incubation at 37°C. The successive dilutions and the minimal plating volume used under our experimental

Table 1

Comparative antimycobacterial activity of various compounds extracted from the tropical flora in Guadeloupe

Compound	Family	Plant	MIC ^a ($\mu\text{g ml}^{-1}$) for					
			<i>M. tuberculosis</i>		<i>M. avium</i>		<i>M. kansasii</i>	
			Bactec	7H11 agar	Bactec	7H11 agar	Bactec	7H11 agar
Pilocarpine	alkaloid	<i>Pilocarpus racemosus</i>	> 100	> 200	> 100	> 200	> 100	> 200
Heraclenol	coumarin	<i>Amyris elemifera</i>	100	200	100	200	> 100	200
Canellal	sesquiterpene	<i>Canella winterana</i>	100	200	> 100	> 200	100	200
Lochnerin	indole alkaloid	<i>Rauwolfia biauriculata</i>	100	> 200	> 100	> 200	> 100	> 200
Ibogaine	indole alkaloid	<i>Tabernaemontana citrifolia</i>	50	50	100	> 200	50	50
Isomeranzin	coumarin	<i>Triphasia trifolia</i>	100	200	> 100	> 200	> 100	> 200
Voacangine	indole alkaloid	<i>Tabernaemontana citrifolia</i>	50	50	50	> 200	100	100
Texalin	oxazole alkaloid	<i>Amyris elemifera</i>	25	25	25	50	25	50
Canella oil	NA ^b	<i>Canella winterana</i>	100	> 200	> 100	> 200	> 100	> 200

^aMICs were determined radiometrically using the Bactec 7H12 broth and the 1% proportion method with Middlebrook 7H11 agar.

^bNot applicable as it was a mixture of various compounds (see text).

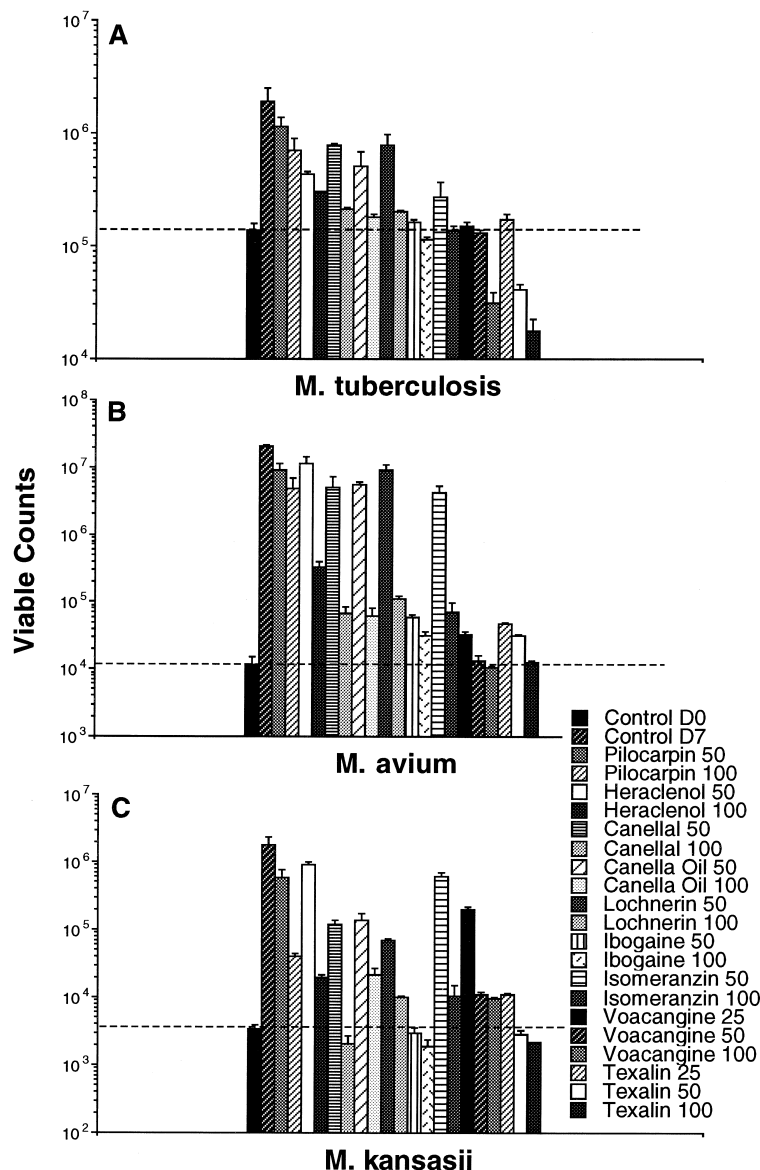


Fig. 3. Effect on bacterial viable counts (mean CFU $\pm$ standard error) of chemically defined compounds extracted from the tropical flora of Guadeloupe. A: *M. tuberculosis*. B: *M. avium*. C: *M. kansasii*. Control D0, control at day 0; Control D7, control at day 7. Numbers after the compounds indicate final concentration in $\mu\text{g ml}^{-1}$ in test Bactec vials while the dotted line represents the initial inoculum added to the Bactec vials at time 0.

conditions avoided any artefactual decrease of bacterial viable counts because of drug carry-over. The results were expressed as mean viable count $\pm$ standard error.

3. Results and discussion

Tuberculosis still results in about 10 million new cases each year with about 3 million deaths. This unfortunate progression of the disease can only be

controlled by following efficient programs of chemoprophylaxis and antibiotherapy [2]. *M. avium*, on the other hand, is mostly an opportunistic infection in AIDS patients, which remains difficult to treat because of the multiple drug resistance of these organisms due to a permeability barrier located in the bacterial cell envelope [10]. The treatment strategy for *M. avium* infections is essentially based on the use of two or more drugs at a time because monotherapy is not efficient enough to significantly reduce the bacterial load, and, in addition, may lead to the development of secondary drug resistance [1]. However, within the context of the search for new drugs and despite the reported activity of plant-derived molecules as antimicrobial [5,6,13–15], antiviral and antiamebic [15], non-amphetaminic central stimulants [16], and anticancer and cytotoxic [13,17] agents, their potential as antimycobacterial agents has not received the necessary attention. Consequently, we decided to investigate the antimycobacterial activity of various chemically defined natural substances (Table 1, Fig. 1), that were extracted from the tropical flora in the Caribbean island of Guadeloupe.

The activity of the above compounds was determined against *M. tuberculosis*, *M. avium* and *M. kansasii* using the Bactec radiometric methodology with 7H12 broth, as well as the 1% proportional method using the 7H11 Middlebrook agar medium. The MIC results are summarized in Table 1, whereas typical radiometric data of MIC determination for voacangine, texalin and ibogaine are illustrated in Fig. 2A,B,C for *M. tuberculosis*, *M. avium*, and *M. kansasii* respectively. As can be seen from the results summarized in Table 1 and Fig. 2, three compounds from the tropical flora of Guadeloupe, namely ibogaine, voacangine and texalin, possessed antimycobacterial activity. Corresponding Bactec versus 7H11 MICs ($\mu\text{g ml}^{-1}$) of these compounds against *M. tuberculosis*, *M. avium*, and *M. kansasii* were, respectively, 50, 100, 50 versus 50, >200, 50 for ibogaine; 50, 50, 100 versus 50, >200, 100 for voacangine; and 25, 25, 25 versus 25, 50, 50 for texalin (Table 1). Cultures from Bactec vials were also plated for viable count determination both at the beginning and at the end of the Bactec experiments for all nine compounds, and these results are illustrated in Fig. 3. A detailed analysis of bacterial viable counts

showed a good correlation between the MICs obtained in 7H12 broth and viable counts.

Although it may be premature to conclude to a direct relationship between the chemical and vegetal families versus antimicrobial activity, it can be concluded that only alkaloids (ibogaine, voacangine, and texalin) showed antimycobacterial activity. It should be underlined that indole alkaloids from Apocynaceae (ibogaine and voacangine) had in common the most developed skeleton of the ibogan type; this observation is interesting as the ibogan skeleton is biosynthesized from a more elementary corynan type skeleton (represented by lochnerin in the present investigation) which was not active against mycobacteria. Lastly, heavily oxygenated compounds such as sesquiterpene dialdehydes and coumarins did not show any activity. We believe that investigations on the structure-modification and structure-activity relationships of ibogaine, voacangine and texalin may be helpful in determining novel targets for future antimycobacterial drug development.

Acknowledgments

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